RT PCR PROTOCOL: BD BIOSCIENCES®

This protocol is adapted from a BDBiosciences protocol by the Gene Expression Lab.
This protocol is for use with BD Bioscience's BD Advantage 2 PCR Enzyme System. For additional technical inquiries, contact Technical Service at 877-232-8995 or www.bdbiosciences.com

BEFORE STARTING THE EXPERIMENT

Use PCR Hood
For ~30 minutes prior to starting procedure, use the UV hood to decontaminate the hood.

RT PCR PROTOCOL

Step A. Thermocycler Conditions

1. Enter the parameters listed in Table 1 into the thermocyler.

Table 1: PCR Program

Step	Deactivation		Anneal/Extension (25 cycles)*		Final Polymerization	Hold:
Temperature	95°C	95°C	60°C	72°C	72°C	4°C
Time (min.)	2:00	0:30	0:30	3:00**	7:00	∞

Step B. Master Mix Procedure

1. Prepare master mix for the number of reactions needed.

Component	1rxn
10X Advantage PCR Buffer(vortex)	2.5ul
50X dNTP Mix	0.5ul
Forward primer	1.0ul
Reward primer	1.0ul
50X Advantage 2 Polymerase system	0.5ul
DEPC-treated water	17.5ul
FINAL VOLUME	23.0ul

2. Mix gently, add 23ul master mix to each well and add Add 2ul cDNA, add 24ul Master Mix
3. Close lid, mix, briefly centrifuge, wait to 1000 rpm, then stop. Insert into Gene Amp 9700 and close lid, run samples.
4. Store at -20α

*25 cycles for multiple-copy genes and med-high abundance of cDNA's. 30-35 cycles for low copy-number genes or rare cDNA's
** If size of gene is >2kb, consider increasing length of annealing phase.

TROUBLESHOOTING

1. No products are observed

- Too few cycles may have been used – try increasing the number of cycles, 3-5 additional cycles at a time.
- The extension time may not have been long enough – if the gene is of a larger size, it may be necessary to increase the extension time.
- It is very possible that no products were observed because the gene target is difficult to amplify. It may be worthwhile to check the GC content of the gene and/or the secondary structure. Both of these are important inhibiting factors in amplification.

2. Multiple products are observed

- If there are too many cycles, there may be nonspecific bands. It may be necessary to reduce the number of cycles.
- Perhaps the primers were not designed optimally. It may be necessary to make new ones.